A SUCCESSFUL METHOD FOR MATURING PHYTOPHAGOUS ALPINE FLIES (DIPTERA: ANTHOMYIIDAE)[1]

Ronald E. Abbott[2]

ABSTRACT: Larvae of alpine flies in genus *Botanophila* Lioy (Diptera: Anthomyiidae) were collected from inflorescences of *Saussurea weberi* Hultén (Asteraceae) and subjected to a staged temperature treatment which successfully induced pupation, then adult emergence.

A problem of identification of many insects having complete metamorphosis is the recognition of different sexual or reproductive forms, or life stages as belonging to the same species. In many groups of insects, the adult form is often required to determine species. Moreover, in many taxa including Diptera, male specimens are essential for providing reliable identification (G.C.D. Griffiths, comm.).

Saussurea weberi Hultén (Asteraceae) is a rare alpine calciphilic thistle in the sunflower family, endemic in Colorado, USA, mostly to the Mosquito Range in the central part of the state. A significant aspect of its biology is the relationship with two species of flies whose larvae regularly predate up to 70% of fruit and seed while still in the flowering head. This degree of predation before seed is dispersed may seriously depress the rate of sexual reproduction within plant populations. Knowing the identity of these flies would contribute to a better understanding of the population dynamics of the plant, and in turn to its conservation.

In 1995 maggots from heads of *S. weberi* were successfully reared to adulthood (Abbott, 1998) and identified as belonging to two closely related and possibly undescribed species of *Botanophila* Lioy (Diptera: Anthomyiidae) (G.C.D. Griffiths, comm.). Following is a description of the method used to mature these flies which might be applicable to the rearing for identification of other alpine/boreal phytophagous Diptera.

METHODS AND RESULTS

An Admiral 2.2 ft^3 refrigerator (model USK02004) was first modified to create a freezer capable of maintaining temperatures close to ca -4°C (24°F), this being the mean minimum soil temperature at ca 15cm (6 in.) expected at the *Kobresia*-dominated site from which maggots were taken (Marr, 1967). Modification was accomplished by detaching the thermostat sensor bulb from the cold plate and moving it a few mm away.

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[2] Post Office Box 1431, Greeley, Colorado, USA, 80632-1431.

Inflorescences of *S. weberi* were collected on September 3, 1995 at Horseshoe Cirque in the Mosquito Range (39° 07' 38" N lat. by 106° 10' 00" W long., elevation 3755 m), approximately 21 km (13 mi) west of Fairplay, Colorado, USA.

About two dozen intact inflorescences were put in a 16 oz. mason jar with its lid loosely screwed on, and this immediately placed in a portable cooler with ice. After a 4 hr trip to the laboratory, the mason jar was transferred directly to an ordinary kitchen refrigerator. By this time, some maggots already had left inflorescence heads and fallen to the jar bottom. Over several days 121 maggots thus issued from heads. A few times each day, emerged larvae were transferred to moist vermiculite in a second 16 oz. jar, where upon they burrowed at once beneath the surface and pupated.

Pupae in the second jar were subjected to the following temperature treatment:

A) 20 to 22°C constantly for 2 weeks
B) 20 to 22°C in daytime, 1 to 4°C at night for 5 days
C) 4°C in daytime, 1°C at night for 3 weeks
D) -6 to -2°C constantly for 28 weeks, 6 days
E) repeat of C, B and A, in that order.

For the cold treatment of stages B, C and E, the mason jar was placed unenclosed, with its lid loosely screwed on, in an ordinary kitchen refrigerator. For stage D, in order to dampen temperature fluctuations, the mason jar with loose lid was first packed in dry vermiculite inside a 4500 cm^3 capacity styrofoam shipping box, which itself had a lid penetrated by air holes. This box was then placed in the Admiral refrigerator. Without opening the door of the Admiral refrigerator, the temperature within was checked a few times each day using an electronic thermometer attached by wire to a sensor probe inside. The refrigerator was opened and adjusted every few to several days to maintain close to -4°C.

At the end of stage E, a total of twelve adult flies of both sexes had emerged over a span of 8 days. These were removed from the jar as they emerged, killed and point-mounted.

DISCUSSION

It is unknown whether or not the 10% emergence rate observed reflects that which normally occurs in the alpine. It is likely, however, that this low observed emergence rate was a result of accumulation of fat reserves by many larvae insufficient to allow survival through the pupation process.

Neither is it known whether or not the procedure can be modified to increase that 10% rate in order to obtain more adult specimens, or speeded up by foreshortening one or more stages of the temperature treatment. It might be simplified, though, by allowing maggots to emerge from plant parts and fall directly onto the vermiculite within which pupation would proceed (G.C.D.

simplified, though, by allowing maggots to emerge from plant parts and fall directly onto the vermiculite within which pupation would proceed (G.C.D. Griffiths, comm.).

Perhaps the most critical aspect of this procedure is the timing of the collection of larvae from the field. If collection is made too early, maggots might not have time to attain a state of physiological preparedness to allow pupation to proceed at all; if collection is made too late, maggots will have already emerged from heads on-site, dropped to the soil and pupated.

Temperature certainly provides important environmental cues to these particular alpine flies throughout their maturation process.

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